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Development of mRNA vaccines against respiratory syncytial virus (RSV)

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ABSTRACT

Respiratory syncytial virus (RSV) is a single-stranded negative-sense RNA virus that is the primary etiologic pathogen of bronchitis and pneumonia in infants and the elderly. Currently, no preventative vaccine has been approved for RSV infection. However, advances in the characterization, and structural resolution, of the RSV surface fusion glycoprotein have revolutionized RSV vaccine development by providing a new target for preventive interventions. In general, six different approaches have been adopted in the development of preventative RSV therapeutics, namely, particle-based vaccines, vector-based vaccines, live-attenuated or chimeric vaccines, subunit vaccines, mRNA vaccines, and monoclonal antibodies. Among these preventive interventions, MVA-BN-RSV, RSVpreF3, RSVpreF, Ad26. RSV.preF, nirsevimab, clesrovimab and mRNA-1345 is being tested in phase 3 clinical trials, and displays the most promising in infant or elderly populations. Accompanied by the huge success of mRNA vaccines in COVID-19, mRNA vaccines have been rapidly developed, with many having entered clinical studies, in which they have demonstrated encouraging results and acceptable safety profiles. In fact, Moderna has received FDA approval, granting fast-track designation for an investigational single-dose mRNA-1345 vaccine against RSV in adults over 60 years of age. Hence, mRNA vaccines may represent a new, more successful, chapter in the continued battle to develop effective preventative measures against RSV. This review discusses the structure, life cycle, and brief history of RSV, while also presenting the current advancements in RSV preventatives, with a focus on the latest progress in RSV mRNA vaccine development. Finally, future prospects for this field are presented.

1. Introduction

Respiratory syncytial virus (RSV) is a single-stranded negative-sense RNA virus, and is the most common cause of acute lower respiratory tract infection in young children [1]. RSV infection leads to increased mucus production and inflammation, resulting in narrowing of the airway, thus posing a serious threat to the physical and mental health of children [1,2]. RSV infection contributes substantially to the global morbidity and mortality burden in children aged 0–60 months, particularly during the first 6 months of life, as well as in low- and middle-income countries [1]. Treatment of RSV infection is largely supportive, with modalities such as bronchodilators, epinephrine, corticosteroids, and hypertonic saline [2]. Meanwhile, no vaccines have proven effective in preventing RSV infection [3]. Nevertheless, various RSV vaccines, including particle-based, vector-based, live-attenuated or chimeric, and subunit vaccines [4], are in various stages of clinical trials.

Traditional vaccines are typically based on live or inactivated attenuated virus, or subunit proteins derived from the pathogens [4]. Although these vaccines produce an effective immune response, they have a number of associated safety concerns [4]. Meanwhile, nucleic acid-based vaccines have the potential to offer the combined safety and efficacy of live attenuated and subunit vaccines. While DNA vaccines can induce simultaneous humoral immunity and cellular immune responses, their protection efficiency is typically low, and nucleic acid may integrate into the host genes [5,6]. Alternatively, mRNA vaccines are safer and simpler as they do not pose a risk of nucleic acid

Abbreviations: CNE, cationic nanoemulsion; COVID-19, Corona Virus Disease 2019; EGFR, epidermal growth factor receptor; ERD, enhanced respiratory disease; FI-RSV, formalin-inactivated RSV; ICAM1, intercellular adhesion molecule 1; LNP, lipid-based nanoparticles; NRM, non-replicating mRNA; NS, non-structural; pre-F, pre-fusion; RSV, Respiratory syncytial virus; SAM, self-amplifying mRNA; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SH, small hydrophobic protein; M, matrix; N, nuclear; P, phosphoproteins; G, glycoproteins; F, fusion; BAL, bronchoalveolar lavage; NETs, neutrophil extracellular traps; IL, interleukin; LRTIs, lower respiratory tract infections; RBD, receptor binding domain; FDA, Food and Drug Administration; HIV, human immunodeficiency virus.

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integration into the host genome and can be naturally degraded in vivo, thus, having a wide application prospect [7].

Currently, due to the Corona Virus Disease 2019 (COVID-19) pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), development of mRNA vaccines has been remarkably rapid [8]. mRNA vaccines can transfer the translation of antigens to the host cell while also facilitating the effective expression of unstable antigens [9]. Additionally, mRNA vaccines boast high antigen expression efficiency, high safety, and strong immunogenicity, while also simultaneously activating humoral and cellular immunity [10,11]. Indeed, since the beginning of the COVID-19 pandemic, mRNA vaccines have become a research hotspot owing to their simple production process, and ability to induce strong immune responses [10]. In particular, the Moderna mRNA vaccine developed against SARS-CoV-2 is capable of inducing a strong and long-lasting antibody response without causing significant adverse events [12].

Recently, mRNA vaccines for various infectious diseases, including human immunodeficiency virus (HIV), influenza, Ebola, Zika, and RSV, have entered the preclinical research, or clinical trial, stage [11]. In particular, several mRNA vaccines against RSV infection are currently in clinical trials. In phase I clinical trials, mRNA-1777 promoted the production of RSV neutralizing antibodies and triggered a strong humoral response [13]. Meanwhile, after further engineering and codon optimization, the mRNA-1345 vaccine showed enhanced immunogenicity. In fact, in a phase I clinical trial, inoculation with mRNA-1345 produced neutralizing antibody titers seven times higher than that with mRNA-1777 [14]. mRNA-1345 has since been approved by the Food and Drug Administration (FDA) for the prevention of RSV infection in people over 60 years of age, and has entered into a multicenter, placebo-controlled phase 2/3 clinical trial [14,15].

This review aims to summarize the current status of RSV vaccine development, focusing on the latest progress in RSV mRNA vaccines development, and provide future prospects for the field of RSV preventative interventions.

2. Respiratory syncytial virus

RSV is a filamentous, enveloped, negative-sense, single-stranded RNA virus that belongs to the genus *Orthopneumovirus* of the family *Pneumoviridae* in the order *Mononegavirales* [3,16]. The RSV genome contains ten genes and is 15.2 kb in length [17] (Fig. 1). It encodes 11 proteins [17], comprising the internal structural proteins, namely, nuclear (N) proteins and matrix (M) protein, as well as non-structural (NS) proteins, including, NS-1 and NS-2, proteins required for functional polymerase complexes, including phosphoproteins (P) and polymerase (L) protein, externally exposed transmembrane glycoproteins, including small hydrophobic proteins (SH), glycoproteins (G), and fusion (F) protein, and regulatory M2 proteins, including M2–1 anti-termination protein and M2–2 protein [2,3]. G glycoprotein functions primarily as an attachment protein, binding virions to target cells by interacting with host cell surface molecules, such as glycosaminoglycan (GAG) and CX3CR1 [3,17]. In addition, G protein is the most variable structural protein in RSV isolates, and its sequences have been used in many epidemiological and evolutionary studies; its variability, in fact, determines the group of RSV antigens (RSV-A and RSV-B) [18]. The F glycoprotein also promotes adhesion, though to a lesser extent than G, its main function being mediation of viral fusion with host cell membranes [3,17]. More specifically, F protein interacts with immobilized heparin, cellular heparin sulfate, intercellular adhesion molecule 1 (ICAM1), epidermal growth factor receptor (EGFR), and nucleolins to promote cell attachment and infection by RSV [3,17]. NS1 and NS2 inhibit apoptosis and type I interferon responses, which contribute to innate immune escape [19]. SH protein is a pentameric ion channel that is widely believed to be involved in delaying apoptosis of infected cells [20], and M protein is a non-glycosylated structural protein located in the inner lobe of the viral envelope, which is related to the cytoplasmic domain of F protein [21]. M2–1 protein contributes to the RSV envelope structure [17,22], while the M2–2 protein governs the switch from transcription to genome replication [23].

2.1. Mechanisms of RSV pathology

In RSV infection, the immune response and viral replication are the primary factors that cause damage to the airway. Given that RSV is reportedly relatively less cytopathic than other respiratory viruses, most injury to the airway may be attributed to the immune response in severe RSV cases [3]. Meanwhile, the data, observed from bronchoalveolar lavage (BAL) fluid, biopsy, and autopsy samples, suggests significant histopathological signs of direct cytopathology caused by viral replication [24,25]. In the following sections we, therefore, discuss

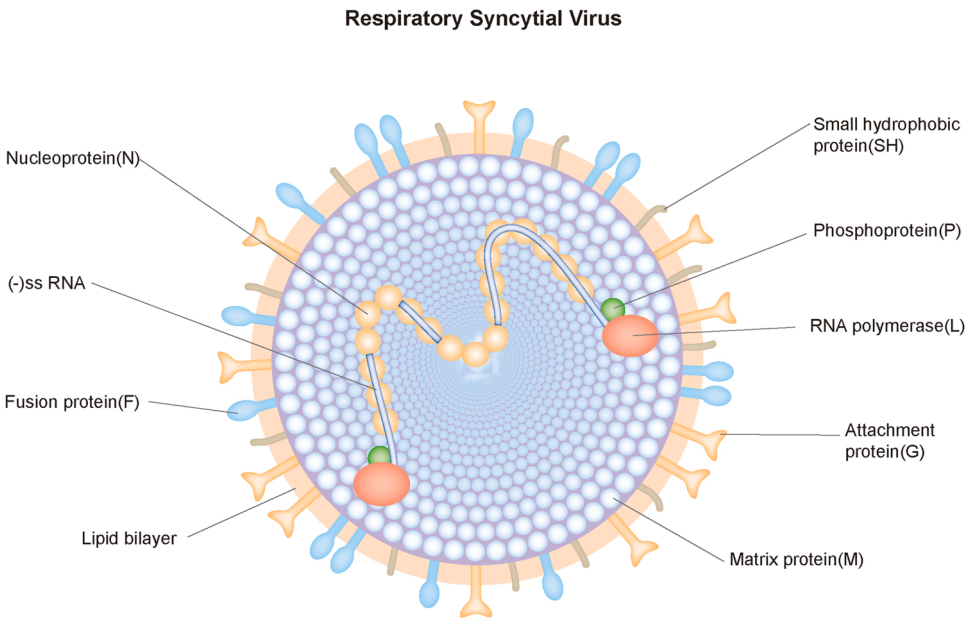

Fig. 1. Respiratory syncytial virus virion. M proteins are present on the inner side of the viral envelope. The G and F glycoproteins, or SH proteins are embedded in the membrane of RSV. RSV RNA is tightly encapsulated by N protein and the L proteins, protein.

RSV-induced airway injury from these two aspects, i.e., viral replication and immune response.

2.1.1. Viral replication

Histopathological analysis of autopsy samples revealed granular intracytoplasmic inclusion bodies—containing mucus, cell debris, and DNA [26,27]—that stained intensely for RSV antigen within the infected cells of severe cases, thus suggesting that viral replication contributes to lung injury in RSV infection [24,25]. RSV replication in bronchial epithelial cells can induce sloughing of these cells into the lower airway bronchioles, causing obstruction [28]. Indeed, in severe cases, the alveoli can become clogged with RSV-infected, and necrotic, debris sloughed from earlier-generation bronchi [25], which can cause acute distal airway obstruction in RSV-infected infants during lower respiratory tract infections [28]. In 2014, a study indicated that NS2 promotes epithelial cell shedding, which contributes to acute obstruction of the distal airways, suggesting that NS2 might be a therapeutic target for reducing the severity of distal airway disease [28].

2.1.2. Immune response

RSV infection often induces neutrophil-intensive inflammation in the respiratory tract during upper and lower respiratory tract infections in infants [29–31]. That is, neutrophils significantly infiltrate the airways of infants with RSV-induced bronchiolitis, accounting for 80% of the total leukocyte population [30,31]. Activated neutrophils can extrude neutrophil extracellular traps (NETs), which are web-like structures, composed of DNA fibers, histones, and antimicrobial proteins, able to entrap bacteria fungi, protozoa, and virus [32]. In RSV infected cases, NETs may be present in bronchoalveolar lavage samples [33,34], and may serve to sequester and inactivate pathogens, including RSV [33,34]. However, abundant NETs formation can also cause airway obstruction during RSV infection [33,35]. Moreover, neutrophils could induce mucus hypersecretion, contributing to acute reversible, and progressive irreversible, airway obstruction [36]. Neutrophil inflammation has also been related to asthma in RSV infection [27, 37, 38]. That is, neutrophils participate in asthma exacerbation by inducing mucus hypersecretion and airway remodeling [39,40]. However, the release of dsDNA from NETs in the airway may also induce asthma exacerbations [30,38]. In addition, neutrophils might sensitize the airways to asthma through mast cell recruitment [38]. Hence, neutrophils play an important role in asthma exacerbation during RSV infection.

Histopathological analysis of autopsy samples has revealed significant eosinophil infiltration in the airways of RSV-infected infants, particularly in severe cases [41]. Indeed, eosinophilia reportedly contributes to RSV disease. Meanwhile, the formalin-fixed RSV (FI-RSV) vaccine has been shown to induce vaccine-enhanced disease, which is closely associated with airway eosinophilic inflammation [42]. Moreover, eosinophilic infiltration is often present in the airway of asthma patients, and has a close association with Th2-mediated allergic asthma [43,44]. In fact, eosinophil expansion in RSV infection is directly related to induction and exacerbation of asthma following RSV infection in early life [45].

Moreover, a direct association has been reported between severe RSV disease in infancy and later chronic wheezing and asthma. More specifically, RSV infection induces a Th2-type dominant response, while suppressing the Th1-type response [31]. The Th2 response, characterized by interleukin (IL)− 5, IL-4, IL-9, IL-6, IL-10, and IL-13 production, is involved in asthma development [31]. However, systemically, IL-10, IL-4, IL-6, and IL-13 levels are also elevated in children with RSV lower respiratory tract infections (LRTIs) [46–50]. Meanwhile, locally, within the respiratory tract, the levels of IL-4, IL-6, IL-9, IL-10, and IL-13 are significantly elevated in the nasal washes [46, 51–55] and in lungs [56–59] of children with RSV LRTIs. Hence, RSV infection skews the immune response away from Th1 toward a Th2-dominant response associated with an increased risk of asthma.

2.2. RSV F protein is an important target for vaccine development

Current vaccine candidates for RSV often target the highly conserved F protein. The conserved structural sequence of the F protein may partially account for the presence of a single RSV serotype [60–62]. Meanwhile, compared to the serum neutralizing antibody titers induced by G and SH proteins, those induced by F protein are significantly higher in RSV-infected people [61–63]. Thus, F protein is considered an important target for RSV vaccine development.

During the process of RSV fusion with host cell membranes, the conformation of F protein changes from an unstable pre-fusion conformation (pre-F) to a stable trimeric conformation (post-F) [17]. Importantly, pre-F-specific antibodies determine the magnitude of RSV neutralizing activity in human sera [61]. Moreover, antibodies that bind to pre-F are more efficient at neutralizing RSV than those shared by both pre-F and post-F [62]. In fact, most neutralizing activity in human sera can be adsorbed with pre-F, whereas post-F removes substantially less [61,63]. Given that vaccination against the pre-F conformation produces an excellent neutralizing antibody response [4, 61, 64] pre-F may be considered a promising target antigen for RSV vaccine development.

The pre-F structure has been resolved, and major antigenic sites have been defined on the basis of structural domains, antibody competition, and sequencing of neutralizing antibody-escape mutants [60, 65–67] (Fig. 2). Indeed, characterization of the pre-F protein has revitalized the development of an RSV vaccine [4,64]. To date, six antigenic epitopes in pre-F (Site I, II, III, IV, V, and φ), and four in post-F (Site I, II, III, and IV), have been reported[60, 68, 69] (Fig. 2). Most of the sites on the membrane-proximal regions of the pre-F head domain (sites II, III, and IV) are retained on the post-F molecule following reconfiguration (Fig. 2). In contrast, the apex of pre-F contains sites Ø and V, which are highly neutralization-sensitive and exclusive to the pre-F conformation [60,68]; the neutralization activity of antigen epitope φ-induced antibodies is much higher than that of other epitopes [2, 60, 62]. For example, antibodies against site Ø bind significantly more efficiently to pre-F, than palivizumab binds to site-II, which is present in both pre-F and post-F. Moreover, antibodies against the antigenic site I, also shared between pre-F and post-F, show weak or no neutralization [62, 70]. Therefore, researchers have proposed that vaccine candidates have a stable site φ [60,62].

Vaccine development of candidates containing the F protein has focused on structure-based engineering approaches to stabilize the pre-F conformation. However, given that F protein can destroy cell membranes, cells do not readily re-express F protein after the initial fusion event [71]. Moreover, certain regions of F protein, such as φ epitope, are not efficiently expressed by a prokaryotic expression system [71]. Moreover, lack of glycosylation modification and disulfide bond formation in *Escherichia coli* protein affects vaccine viability [71]. Therefore, a eukaryotic expression system would be more suitable for the production of a human subunit vaccine [71]. Development of protein engineering technology is particularly important as the conformational instability of pre-F limits its purification and expression [72]. Nevertheless, in a phase I clinical trial, the subunit vaccine candidate DS-CAV1, which targets RSV pre-F, was found to increase serum neutralizing active antibody titers by more than 10-fold [72]. Meanwhile, the newly developed mRNA vaccine skillfully avoids the complex protein structure design and directly uses the host cell system to express pre-F, thus providing a novel strategy for RSV vaccine design.

2.3. Journey to an RSV vaccine

RSV vaccine development began shortly after the virus was first identified in humans in 1957 [3]. In the 1960 s, an FI-RSV vaccine was developed, which then underwent clinical trials in infants [73]. The vaccine virus was produced by cell culture, inactivated with formalin, and mixed with alum as an adjuvant [68]. However, FI-RSV was found to mostly express the post-F rather than pre-F conformation [68]. The

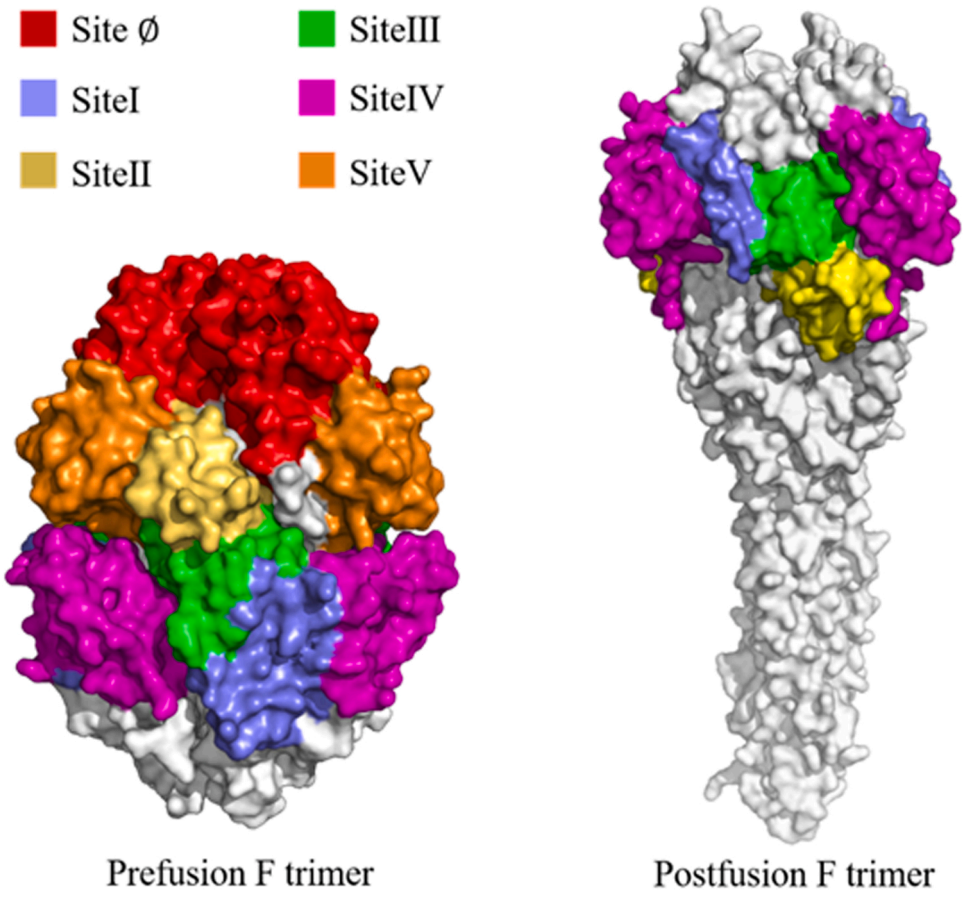

Fig. 2. RSV F structures and antigenic sites. Pre-fusion and post-fusion RSV F structures are shown. Antigenic sites are displayed in different colors: site Ø, red; site I, cyan; site II, earthy yellow; site III, green; site IV, pink; and site V are orange.(For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

vaccine not only failed to protect young seronegative infants against RSV disease, but also resulted in severe enhanced respiratory disease (ERD), leading to hospitalization of 80% of the tested infants and two deaths [73]. The ERD was caused, in part, by formalin-mediated destruction of neutralizing epitopes in the vaccine preparation [74]. FI-RSV injection could lead to immunologic derangement, represented by an increased number of $CD4^+$ T cells and Th2 cytokine levels; meanwhile, the number of $CD8^+$ T cells and Th1 cytokine levels were reduced, possibly also contributing to ERD [74]. Hence, the potential risk of ERD development must be considered when developing inactivated and protein vaccines in seronegative children [68]. In fact, the adverse events associated with the FI-RSV have delayed the

	Live-attenuated	Subunit	Vector-based	Particle-based	Monoclonal antibody	mRNA
Phase 3	None	1. RSVpreF3 2. RSVpre-F	1. MVA-BN-RSV 2. Ad26.RSV.preF	None	1. Nirsevimab 2. Clesrovimab	mRNA-1345
Phase 2	RSV/ΔNS2/Δ1313/I1314L	MEDI7510	None	None	Narsyn	None
Phase 1	1. LID ΔM2-2 2. LIDcpΔM2-2 3. LID/ΔM2-2/10 4. D46/NS2/N/ΔM2-2-HindIII30s 5. RSVΔG 6. rA2cp248/404ΔSH 7. rA2cp248/404/1030ΔSH 8. MEDI-599	1. DS-Cav1 2. RSV F vaccine 3. DPX-RSV	1. MVA-RSV 2. PanAd3-RSV 3. ChAd155-RSV	IVX-121 V306-VLP	RSM01	mRNA-1777

Fig. 3. Overview of the RSV vaccine candidates and monoclonal antibodies in clinical trials.

development of an RSV vaccine for decades [75]. Nevertheless, currently, there are five types of RSV vaccines under development, namely particle-based, vector-based, live-attenuated or chimeric, subunit vaccines, and mRNA vaccines (Fig. 3).

2.3.1. Live-attenuated vaccine candidates

Live-attenuated vaccine candidates aim to activate a local mucosal antibody and cellular response by mimicking natural infection, while being attenuated for reduced virulence. Live-attenuated vaccine candidates have been considered safe for clinical evaluation in children as they are not expected to cause ERD [76]. However, given that some live attenuated RSV vaccine candidates have been insufficiently attenuated, whereas others are highly attenuated but insufficiently immunogenic [77–79], achieving the balance of attenuation and immunogenicity of RSV vaccine candidates in naive children and infants has proven difficult [3, 4, 75]. Reverse genetics techniques provide a means to address this concern [4, 75, 80].

The RSV M2–2 gene mediates the transition from transcription to RNA replication of RSV [3,17]; its deletion can be used to develop an attenuated vaccine with enhanced immunogenicity [23]. Compared to wild-type RSV, the RSV ΔM2–2 mutant decreases genome replication while substantially increasing synthesis of viral proteins, including the major neutralization and protective antigens [23]. In a chimpanzee study, RSV ΔM2–2 mutant restricted RSV replication and induced substantial neutralizing serum antibody responses [81]. Subsequently, in 2015, a clinical trial was conducted to evaluate the effectiveness of an RSV MEDI ΔM2–2 vaccine candidate in adults and children. The phase 1 results showed that the replication of RSV MEDI ΔM2–2 was highly restricted in RSV-seronegative children [80]. However, neutralizing antibody titers were boosted against RSV by 4-fold and a $\geq$ 4-fold increase in anti-F antibody titers was reported in vaccine recipients [80]. In 2018, other trails (NCT02237209 and NCT02040831) evaluated the safety and immunogenicity of the LID ΔM2–2 vaccine in RSV-seronegative children aged 6–24 months [82]. The results showed 90% of vaccinees had a $\geq$ 4-fold rise in serum neutralizing antibodies and anti-F IgG antibodies [82]. Hence, LID ΔM2–2 appeared to have acceptable infectivity and immunogenicity [82]. In 2019, two trails (NCT02890381 and NCT02948127) evaluated the effectiveness of another RSV candidate vaccine (LIDcpΔM2–2) that is attenuated through deletion of M2–2 and five cold-passage mutations [83]. Results showed only 45% of vaccinees had a $\geq$ 4-fold increase in serum-neutralizing antibodies [83], while both vaccinees (64%) and placebo recipients (100%) developed respiratory symptoms or fever. In 2020, two trails (NCT02952339 and NCT02794870) evaluated the safety and immunogenicity of another RSV candidate vaccine (LID/ΔM2–2/1030 s) in RSV-seronegative children (aged 6–24 months) [84]. The virulence of LID/ΔM2–2/1030 s was attenuated by M2–2 deletion, and genetic stabilization temperature-sensitivity mutation 1030 s in the RSV polymerase protein [84]. The results showed serum RSV-neutralizing antibody, and anti-RSV IgG titers increased by $\geq$ 4-fold in 95% and 100% of vaccinees, respectively [84]. Respiratory symptoms and fever were common in vaccinees and placebo recipients [84]. Thus, LID/ΔM2–2/1030 s exhibited excellent infectivity and induced durable immunity [84]. In 2020, the safety and immunogenicity of RSV candidate vaccine D46/NS2/N/ΔM2–2-*Hin*dIII was evaluated in RSV-seronegative children aged 6–24 months (NCT03102034 and NCT03099291) [85]; the serum RSV-neutralizing antibody, and anti-RSV fusion immunoglobulin G titers increased by $\geq$ 4-fold in vaccinees. Mild upper respiratory tract symptoms and/or fever occurred in vaccinees (76%) and placebo recipients (18%) [85]. In conclusion, D46/NS2/N/ΔM2–2-*Hin*dIII had excellent infectivity and immunogenicity, and further evaluation is encouraged [85].

G protein is major surface glycoprotein of RSV, however, is not essential for RSV replication. Nevertheless, the absence of G-protein can reduce RSV replication competence [86], which may achieve attenuated virulence. Indeed, in the absence of the G protein an effective immune response is still induced due to the F protein serving as the major antigen site [87]. Several preclinical studies have been conducted to evaluate whether RSV lacking the G protein can be used as live-attenuated vaccine candidates. In 2002, a recombinant bovine RSV deletion mutant lacking the G gene induced neutralizing antibodies, was safe, and provided protection against RSV challenge [88]. In 2004, a recombinant human RSV lacking the G gene elicited serum neutralization in a mouse model [89]. In 2010, recombinant RSV lacking the G protein was highly attenuated and conferred long lasting protection against wild-type RSV challenge in a cotton rat model [90]. In 2020, a first-in-human study was conducted to evaluate the safety, tolerability, viral shedding, and immunogenicity of RSVΔG in healthy adult volunteers [87]. The virulence of RSVΔG was sufficiently attenuated, and RSVΔG was safe and well-tolerated in volunteers. However, no significant induction of antibodies was noted after RSVΔG vaccination [87].

The virulence of recombinant RSV bearing a deletion of either the NS2 or SH gene is reportedly attenuated in chimpanzees [91]. Although loss of the SH gene did not noticeably affect RNA replication, the expression of G, F, and M2 mRNAs was increased [92]. When administered intranasally to mice, the SH-minus and wild-type RSV were similarly immunogenic and effective in inducing resistance to viral challenge [92]. A clinical trial evaluating two live attenuated RSV vaccine candidates, (rA2cp248/404ΔSH and rA2cp248/404/1030ΔSH) in adults and children [93] reported highly attenuated virulence of the vaccines that were well tolerated and immunogenic in RSV-seronegative children; however, only 44% of infants who received the vaccine had detectable antibody responses [93]. Meanwhile, MEDI-599—SH deletion vaccine—was associated with a higher incidence of medically attended lower respiratory illness [94] and, thus, must be further evaluated for safety.

Recombinant RSV bearing an NS2 gene deletion (rA2ΔNS2) is highly attenuated in the lower respiratory tract of chimpanzees, and induces significant resistance to wild-type RSV challenge [91]. Thus, a candidate vaccine was developed with an NS2 deletion and deletion of codon 1313 in the polymerase (L) gene [28]. In 2013, a recombinant live-attenuated vaccine with combined Δ1313 and NS2 deletion proved to be attenuated and immunogenic in nonhuman primates [95]. Subsequently, a single intranasal dose of RSV/ΔNS2/Δ1313/I1314L was evaluated in a double-blind, placebo-controlled trial in RSV-seropositive children. RSV/ΔNS2/Δ1313/I1314L was well tolerated, infectious, and immunogenic warranting further evaluation [96].

2.3.2. Subunit vaccine candidates

Owing to concerns of ERD associated with protein-based vaccines, subunit vaccines are only intended for pregnant persons and elderly individuals [4]. Moreover, the current subunit vaccines use stabilized pre-F as the main antigen [67]. The DS-Cav1 subunit vaccine can elicit unprecedented levels of neutralizing antibody activity due to the presence of several neutralization-sensitive antigenic sites on the trimer apex [60]. A previous study reported high neutralizing antibody titers elicited following immunization with DS-Cav1, compared with post-F, that were 70- and 80-fold higher for subtypes A and B respectively, in rhesus macaques [60,97]. The findings about pre-F DS-Cav1 was considered to be one of the top scientific breakthroughs of 2013 [98]. In 2021, phase 1 clinical trial results for DS-Cav1 reported a more than 10-fold boost in serum neutralizing activity compared to antibodies targeting pre-F protein [72]. A phase 1, randomized, open-label, dose-escalation clinical trial showed that, DS-Cav1 vaccination elicits a robust boost in RSV F-specific antibodies and neutralizing activity, which are sustained above baseline for at least 44 weeks. Moreover, DS-Cav1 vaccination is well tolerated with acceptable safety. Hence, a single low-dose of DS-Cav1 vaccination might be sustainable for an entire RSV season [99].

RSVpre-F (PF-06928316) uses a stabilized pre-F protein as the vaccine antigen. In 2017, a randomized, controlled, observer-blinded phase 1 study was conducted to evaluate the safety and immunogenicity of RSVpre-F vaccine with or without alum adjuvant in healthy men. The

result showed 30 μg RSV-PreF/alum, 60 μg RSV-PreF/alum, and 60 μg RSV-PreF/nonadjuvant groups elicited the highest RSV neutralizing antibody responses, with an acceptable adverse event profile [100]. In 2018, two observer-blinded, controlled studies assessed the immunogenicity and safety of RSV-PreF vaccine in healthy, nonpregnant 18–45 year-old persons. RSV-PreF vaccination elicited a robust boost in RSV-A-neutralizing antibody that was 3.1- to 3.9-fold higher than the control groups. No serious adverse events were considered vaccine related [101]. In 2019, another phase 2 study evaluated the immunogenicity and safety of RSV-PreF vaccine in healthy nonpregnant persons aged 18–45 years. The SV-PreF vaccine was well-tolerated and induced RSV-A and RSV-B neutralizing antibodies and palivizumab competing antibodies [102]. In 2022, a phase 1/2 study evaluated the RSVpreF vaccine with antigens from RSV subgroups A and B in 18–49-year-old adults. RSVpreF vaccine was safe and well tolerated and elicited 10.6–16.9-fold increases in neutralizing antibodies for RSV A and 10.3–19.8-fold for RSV B at 1 month postvaccination, and 3.9–5.2-fold and 3.7–5.1-fold, at 12 months postvaccination, respectively [103]. Also in 2022, a phase 2b trial evaluated the efficacy, immunogenicity, and safety of RSVpreF vaccine in pregnant persons and their infants [64]. The vaccine elicited neutralizing antibody responses in participants with efficient transplacental transfer and without evident safety concerns [64]. Recently, a phase 1/2 study evaluating the safety and immunogenicity of RSVpreF vaccine candidate with/without adjuvant in adults 65–85 years reported that the formulations were well tolerated and elicited robust neutralizing responses in older adults; however, CpG/Al(OH)3 did not further enhance the responses. Most recently, a human challenge trial reported that RSVpreF vaccine is effective against symptomatic RSV infection and viral shedding with no evident safety concerns [104]. The phase 3 clinical trial is conducting to evaluate the efficacy and safety of maternal immunization with RSVpreF against medically attended lower respiratory tract illness in infants [105]. RSVpreF3 (GSK3888550A) is another pre-F subunit vaccine candidate designed by GSK that shares the same primary sequence of RSVpre-F (PF-06928316) and is stabilized in the pre-F state. In a preclinical study, this vaccine was well-tolerated locally and systemically with an acceptable safety profile and no adverse effects on female reproductive function or on the pre- and postnatal growth and development of offspring [106]. In a first-in-human, placebo-controlled study, the RSVPreF3 vaccine candidate was well tolerated and immunogenic in nonpregnant persons. Moreover, it induced 8- to 14-fold and 12- to 21-fold increases in anti-RSV A and B neutralizing antibody titers on day 8, which remained 5- to 6-fold and 6- to 8-fold higher on day 91, compared with pre-vaccination values [107]. Recently a phase I/II randomized clinical trial evaluated the safety and immunogenicity of RSVPreF3 in older adults and reported an acceptable safety profile as well as a robust boost in RSVPreF3-specific IgG and RSV-A neutralizing antibody responses. Moreover, compared to pre-vaccination, RSVPreF3 increased the frequency of polyfunctional $CD4^+$ T-cells [108]. Meanwhile, a phase 3 study evaluating a maternal RSVPreF3 vaccine reported induced durable immune responses with anti-RSV antibody levels remaining elevated for 6 months after birth [109].

MEDI7510, a post-F protein vaccine candidate, has also been evaluated in several clinical trials [110]. For instance, a double-blind phase 1 study evaluated MEDI7510 with or without an adjuvant in adults aged $\geq$ 60 years and reported that 50% of participants in the 80 μg dose + adjuvant group exhibited a $\geq$ 3-fold geometric mean fold increase in RSV neutralizing antibody titers. In fact, all tested doses induced a $\geq$ 3-fold increase in anti-F IgG antibody titers. However, immune responses were antigen dose-dependent, and the inclusion of adjuvant increased both humoral and cellular immune responses [110], and the safety profile was acceptable for further development. In 2017, a phase 2b study evaluated RSV post-F protein with glucopyranosyl lipid adjuvant in adults aged $\geq$ 60 years. However, efficacy was not observed in this population as immunogenicity was observed, however, was insufficient to protect older adults from RSV illness [111]. Also in 2017, the second phase 1 study of an RSV vaccine containing adjuvanted RSV fusion protein (sF) was conducted in adults aged $\geq$ 60 years. Humoral and cellular immune responses were observed with the 120 μg sF plus 5.0 μg GLA formulation inducing the highest responses in all participants [112]. In 2019, a RSV post-F protein vaccine was developed to protect young infants via maternal immunization, and a first-in-human, phase I observer-blind study evaluated the associated safety and immunogenicity. This vaccine was well-tolerated with no serious adverse events reported and effectively enhanced immune responses that lasted through 6 months of follow-up [113].

DPX-RSV is a unique vaccine as it does not induce neutralizing antibodies but aims at elimination of infected cells. That is, the extracellular domain of the SH protein of RSV subgroup A serves as the antigen, [114]. In 2018, a first-in-human RCT evaluated the safety and immunogenicity of this novel vaccine strategy formulated with either the lipid and oil-based vaccine platform DepoVax (DPX-RSV[A]) or alum (RSV[A]-Alum), in healthy adults aged 50–64 years [115]. Robust anti-SH-specific immune responses were demonstrated in the DPX-RSV(A) 10-μg and 25-μg groups (approximately 10-fold and 100-fold higher than that of the placebo on days 56 and 236, respectively); responses were sustained in the DPX-RSV(A) 25-μg group on day 42 1. In contrast, responses to the RSV(A)-Alum vaccines were very low. Moreover, there was no indication that the vaccine was unsafe with mild pain, drowsiness, and muscles aches reported as the most common solicited adverse events (AEs). Importantly, the frequencies of the AEs did not increase after dose 2 [115].

2.3.3. *Vector-based vaccine candidates*

The RSV vector-based vaccines contain portions of the RSV protein-encoding genome inserted via an innocuous adenovirus or another non-pathogenic virus vector-like modified vaccine Ankara (MVA), i.e., a replication-defective smallpox viral vector [116]. Five vector-based vaccines are currently in clinical development [4].

The chimpanzee adenovirus, PanAd3-RSV, and modified vaccinia virus Ankara, MVA-RSV, are replication-defective viral vectors encoding the RSV fusion (F), nucleocapsid (N), and matrix (M2–1) proteins for the induction of humoral and cellular responses. PanAd3-RSV and MVA-RSV, both encode the F, N, and M2–1 proteins of RSV, and use adenovirus and MVA as a vector, respectively [117]. In 2015, a study evaluated the safety, immunogenicity, and efficacy of PanAd3-RSV and MVA-RSV in rodents and nonhuman primates revealing that a single intranasal or intramuscular administration protected BALB/c mice and cotton rats against RSV replication in the lungs. However, only intranasal vaccination prevented infection in the upper respiratory tract. Meanwhile, PanAd3-RSV and MVA-RSV also elicited high neutralizing antibody titers and broad T-cell responses in nonhuman primates. In addition, intranasal vaccination elicited mucosal IgA against the F protein. Hence, PanAd3-RSV and MVA-RSV induced potent cellular and humoral responses [116]. Also in 2015, an open-label, dose escalation, phase 1 clinical trial was performed to investigate for safety and immunogenicity of PanAd3-RSV and MVA-RSV in 42 healthy adults. The vaccines proved safe and well tolerated with no vaccine-related serious adverse events reported. Moreover, PanAd3-RSV vaccination via intramuscular or intranasal increased the RSV neutralizing antibody and circulating anti-F IgG titers. Intramuscular PanAd3-RSV also increased RSV-specific T cell responses, and efficiently boosted RSV-specific T cell responses. Hence, these vaccine candidates are safe and immunogenic in healthy adults [117], however, further clinical evaluation is needed. In 2019, a first-in-man vaccine trial to evaluate the safety and immunogenicity of MVA-RSV and PanAd3-RSV in healthy older adults aged 60–75 years was conducted. The mean fold-changes of serum RSV-neutralizing antibody, F-specific IgG antibody, and expansion of $CD4^+$/$CD8^+$ IFNγ-producing T-cells were comparable to the results reported for younger healthy adults who received the same vaccine [118]. That is, the RSV-specific immune responses to vaccination did not appear to be attenuated and MVA-RSV and PanAd3-RSV were found to

be safe and well tolerated in older adults [118]. Hence, the PanAd3-RSV and MVA-RSV vaccine candidates can induce RSV-specific humoral and cellular immunity in older adults, however, further assessment of the protection provided against severe disease is needed [118].

MVA-BN-RSV is another vector-based vaccine candidate based on the MVA-BN backbone, that encodes F, G, N and M2 protein [119,120]. In 2020, a first-in-human phase I trial with MVA-BN-RSV assessed safety, reactogenicity and immune responses of MVA-BN-RSV in healthy younger (18–49 years of age) and older (50–65 years of age) adult participants [119]. The MVA-BN-RSV vaccine candidate was safe and well tolerated and effectively induced robust 3.8-fold increased T cell responses, and 2-fold increases in humoral responses against A and B RSV subtypes. Thus, MVA-BN-RSV can induce broad cellular and humoral immune responses [119]. In 2021, a phase 2 clinical study investigating the immune response of MVA-BN-RSV in adults aged $\geq$ 55 years sought to identify the optimal MVA-BN-RSV dose and vaccination schedule. A single dose vaccination was found to increase the levels of neutralizing and total antibodies by 1.6–3.4-fold. Moreover, MVA-BN-RSV vaccination induced a broad Th1-biased cellular immune response with 5.4- to 9.7-fold increases. Additionally, the induced antibody responses remained above baseline for 6 months; a 12-month booster dose elicited antibody and T-cell responses with 2.8-fold increase from pre-boost levels. Hence, MVA-BN-RSV can elicit a broad and long-lasting immune response [120]. Nowadays, a phase 3 clinical trial is conducting to evaluate the efficacy and safety in adults > =60 years of age [121].

Ad26. RSV.FA2 is a vector-based vaccine candidate that uses Ad26 as a vector, and encodes the wildtype RSV F transgene. Preclinical studies showed that Ad26. RSV.FA2 can elicit strong and long-lasting humoral and cellular responses in adult mice and cotton rats [122]. Indeed, previous studies reported that vaccination against the pre-F conformation can produce an excellent neutralizing antibody response [4, 61, 64]. Therefore, a new a vector-based vaccine candidate, named Ad26. RSV.preF, was designed using the Ad26 vector and encoding the full-length RSV F protein with amino acid substitutions that stabilize the RSV F protein in its prefusion conformation [123]. Preclinical studies showed that Ad26. RSV.preF induces robust cellular immune responses in adult and neonatal mice, characterized by IFN-γ-producing $CD4^+$ T and $CD8^+$ T cells, with a profound Th1 bias. Moreover, Ad26. RSV.preF induces robust and durable humoral immunity in neonatal mice, characterized by IgG2a-dominated RSV F-binding antibodies, and high and stable virus-neutralizing titers [123]. In 2020, a phase 1 clinical trial was performed to evaluate Ad26. RSV.preF in healthy adults aged $\geq$ 60 years. Ad26. RSV.preF increased neutralizing antibodies compared with baseline (approximately 2.5-fold in the low dose group, and 3-fold in high-dose group). Titers of Pre-F-specific antibody, and frequencies of F-specific interferon γ-secreting T cells also increased substantially after the first vaccination compared to baseline. These immune responses lasted up to 2 years after the first immunization, whereas a second immunization boosted these immune responses. Hence, Ad26. RSV.preF has been proven was safe and capable of sustaining immune responses in older adults [124]. In 2021, a phase 2a, double-blind, placebo-controlled study was conducted to assess co-administration of Ad26. RSV.preF, with a seasonal influenza vaccine (Fluarix) in older adults. This formulation was safe and did not interfere with immune responses [125]. In 2022, a double-blind, placebo-controlled study aimed to assess the potential of Ad26. RSV.preF to protect against RSV infection and disease in an RSV human challenge model. The results showed that Ad26. RSV.preF could protect humans from RSV infection through immunization and help to defend against natural RSV infection [126].

In 2020, ChAd155-RSV, a new vector-based vaccine candidate was developed for pediatric use. This candidate uses Chimpanzee-derived adenoviruses as the vector, and encodes F, N and M2–1 proteins. A first-in-human clinical trial was conducted to assess the safety, reactogenicity, and immunogenicity of ChAd155-RSV in healthy 18–45-year-old adults. ChAd155-RSV reportedly had an acceptable safety profile and elicited RSV-specific humoral and cellular immune responses in adults previously naturally exposed to RSV [127]. Moreover, the results from a recent preclinical study confirmed that ChAd155-RSV is well-tolerated locally and systemically [128].

2.3.4. Particle-based vaccine candidates

Particle-based vaccines harness the immunogenicity of displaying multiple antigens via particle assembly [109]. More specifically, particle-based vaccine candidates use aggregates of a modified stabilized F protein [4]. This RSV F nanoparticle-based vaccine platform has been evaluated in several clinical studies. For example, IVX-121 is a particle-based vaccine candidate that employs a self-assembling synthetic virus-like particle platform technology to deliver 20 copies of stabilized trimeric pre-F proteins [109,129]. In a preclinical study, this vaccine candidate elicited excellent neutralizing antibody responses 10-fold higher than trimeric DS-Cav1 in mice and nonhuman primates [129]. In 2022, a phase 1 trial for IVX-121 reported a robust immunologic response, consistent across young and older adults, as well as a favorable tolerability profile [130].

V306-VLP, another particle-based vaccine candidate, uses a synthetic virus-like particle to display a site 2 F protein epitope. In preclinical study, a V306 construct was found to elicit strong, long-lasting RSV-neutralizing antibody responses in mice and rabbits that protected mice from RSV infection [131]. Meanwhile, V-306 has also been shown to be safe and efficacious in enhancing RSV preexisting immunity in mice [132]. V-306 aims to boost pre-existing immunity in vulnerable populations, including young infants via pregnant persons, and the elderly [132]. A phase 1 trial is currently underway in healthy women to evaluate the safety and immunogenicity of a candidate vaccine against RSV.

Although these particle-based vaccines are currently in early development, data shows that they can elicit a robust immune response, and represent promising candidates for pregnant persons and the elderly.

2.3.5. Monoclonal antibodies

Recently, development of preventive interventions for RSV has expanded rapidly (Fig. 3). In the 1990 s, a polyclonal immunoglobulin preparation with high anti-RSV neutralizing activity, named RSV-IVIG, was the first licensed products for RSV [133,134]. RSV-IVIG was effective in reducing the incidence, and total days, of RSV hospitalization in infants [134]. In 1998, RSV-IVIG was replaced by the second approved product palivizumab, a humanized monoclonal antibody that targets RSV F glycoprotein. Palivizumab can prevent RSV-induced severe lower respiratory tract infection in children born prematurely with congenital heart disease [135]. Moreover, it has an excellent safety profile and demonstrates a reduction of 39% to ~80% of RSV hospitalizations in preterm infants with, and without, chronic lung disease, respectively [134]. Indeed, palivizumab remains the only licensed preventive intervention against RSV.

Recent data have shown that nirsevimab (also named MEDI-8897), a human monoclonal antibody that targets site Ø of the F protein (Fig. 2), is a promising candidate for RSV prevention [109]. In 2017, Zhu et al. reported that MEDI-8897 effectively neutralizes a wide array of RSV A and B viruses and protects cotton rats at lower doses than palivizumab [136]. Moreover, MEDI-8897 can persist in the circulation; infants could be given a single dose of MEDI-8897 and be protected for the entirety of the RSV season [136]. A Phase 1b/2a randomized, double-blind, placebo-controlled, dose-escalation study showed that MEDI8897 had an acceptable safety profile in healthy preterm infants [137]. On day 151, 87% of infants in the 50 mg group had serum concentrations above the 90% effective concentration target level of 6.8 μg/mL, and 90% showed a $\geq$ 4-fold increase from baseline in serum RSV-neutralizing antibody levels. That is, RSV-neutralizing activity supports protection from RSV for the duration of a typical 5-month season after a single 50 mg intramuscular dose [137]. Moreover, a clinical trial showed that MEDI8897 had a favorable safety profile and was well tolerated in healthy adults

[138]. In 2020, a phase 2 trial aimed to evaluate the efficacy, safety, pharmacokinetics, and antidrug antibody response for MEDI8897 in healthy preterm infants between 29 and 35 weeks' gestation and entering their first RSV season [139]. The incidence of medically attended RSV-associated lower respiratory tract infection was 70.1% lower with nirsevimab prophylaxis than with placebo and the incidence of hospitalization for RSV-associated lower respiratory tract infection was 78.4% lower with nirsevimab than with placebo. In fact, a single injection of nirsevimab resulted in fewer medically attended RSV-associated lower respiratory tract infections and hospitalizations than placebo throughout the RSV season in healthy preterm infants [139]. In 2022, a phase 3 trial evaluated the efficacy, safety, pharmacokinetics, and antidrug antibody response for MEDI8897 in healthy late preterm and term infants ($\geq$ 35 weeks' gestation) and entering their first RSV season [140]. The primary efficacy endpoint of medically attended lower respiratory tract infection associated with RSV occurring up to 150 days after the injection was approximately 75% lower in the nirsevimab group versus placebo [140]. However, the number of hospitalizations for lower respiratory tract infection associated with RSV did not differ significantly between the groups [140]. Hence, a single injection of nirsevimab administered before the RSV season protected healthy late-preterm and term infants from medically attended RSV-associated lower respiratory tract infection [140]. In 2022, nirsevimab was reported as a promising therapy for RSV [140,141].

Clesrovimab (also named MK-1654), is an extended half-life monoclonal antibody that targets F protein site IV (Fig. 2) [142]. A preclinical study showed that RB1 could neutralize diverse RSV clinical isolates in vitro, and protect against RSV infection in a cotton rat challenge model [142]. In 2021, a phase 1 randomized, double-blind, placebo-controlled trial was conducted to assess the safety, tolerability, and pharmacokinetics of MK-1654 in healthy adults. A single dose of MK-1654 effectively elevated RSV serum-neutralizing antibody titers; the antibodies exhibited a half-life of 73–88 days. Moreover, MK-1654 had an acceptable safety profile with no associated adverse events [143]. In 2022, another phase I study reported that MK-1654 was generally well-tolerated and safe in Japanese adults. The RSV serum neutralizing antibody titers increased in a dose-dependent manner among participants who received MK-1654, and the antibodies exhibited a half-life of 76–91 days [144]. Currently, Clesrovimab is in phase 2b/3 trials (NCT04767373) and phase 3 trials (NCT04938830). These studies aim to evaluate the efficacy and safety of clesrovimab in healthy pre-term and full-term infants.

3. mRNA vaccines

3.1. Development of mRNA vaccines

Nucleic acid vaccines based on mRNA were conceived more than three decades ago; however, mRNA was not used as a therapeutic tool owing to concerns regarding stability, poor efficacy, and excessive immunostimulation. In 1990, the first report on mRNA vaccines, showed that RNA expression vectors, injected into mouse skeletal muscle, could express proteins in vivo, with no special delivery system required [145]. However, due to instability and high immunogenicity of the mRNA, further development and application were halted [146]. In 2005, Karikó and colleagues found that mRNA synthesized using modified uridine could avoid recognition and degradation by the immune system, leading to greatly improved mRNA stability and immunogenicity in vivo, thereby inaugurating a new era in mRNA vaccine [147]. Indeed, the associated study has been hailed as a cornerstone insight that directly led to the design and delivery of mRNA vaccines against COVID-19 [148]. Moreover, lipid nanoencapsulation can protect mRNA from the RNAse enzyme, which is present in abundance throughout the body and can disintegrate mRNA almost instantly upon contact [149]. Subsequently, researchers have continued to improve the delivery vector, nucleic acid chain stability, and antigen expression efficiency, while reducing allergic reactions associated with mRNA vaccines. Currently, two types of mRNA vaccines have been reported, namely non-replicating mRNA (NRM) and self-amplifying mRNA (SAM), which are derived from plus-strand RNA viruses. NRM is a complete mRNA-encoding antigen protein transcribed in vitro and has the advantages of simple structure, short RNA sequence, no additional coding protein, and no unrelated immune responses [47]. Meanwhile, SAM vaccines are virus-based vaccines that replicate and express themselves in cells. They not only contains the basic structure of NRM, but also contain nucleic acid sequences similar to that in replicating viruses; hence they can be replicated in cells to improve the expression of antigenic proteins [58]. SAM vaccines reportedly produces higher antigen expression levels compared to NRM vaccines, with a much lower effective dose [59].

Good delivery vectors are also important for the effectiveness of mRNA vaccines. Due to the large relative molecular weight and negative charge of mRNA, it does not readily cross the phospholipid membrane bilayer structure [150]. In addition, mRNA vaccines are easily phagocytosed by cells of the innate immune system and degraded by nucleases [150]. Although delivery vectors can protect mRNA from potential digestion by ribonuclease and provide effective uptake by target cells, they must also have high safety profiles and stability [64]. Initially, SAM vaccines used viruses as vectors, which presented inherent immunogenicity risks in delivering mRNA, limited payload packaging capacity, and required difficult production processes [151]. In contrast, non-viral vectors can effectively protect the loaded mRNA from the influence of the external environment and nucleases, while generally having lower immunogenicity, higher safety, relatively convenient design and synthesis, easy production, low cost, and repeatable drug delivery [11,148]. To date, a number of innovative material-based solutions have been developed to serve as non-viral vectors, including lipid-based nanoparticles (LNPs), polyplexes and polymeric nanoparticles, and various peptides [11]. LNPs are the most clinically advanced mRNA delivery vectors and were adopted in all SARS-COV-2 mRNA vaccines that are either currently under development or have been approved for clinical use. LNPs boast the advantages of easy preparation, modularization, biocompatibility, and large mRNA payload capacity [11]. LNPs comprise ionizable lipids, cholesterol, helper phospholipids, and polyethylene glycol lipids, which together encapsulate and protect the fragile mRNA core [11]. Polyplexes and polymeric nanoparticles, although clinically less advanced than LNPs, have advantages similar to lipids and can deliver mRNA efficiently [152]. The cationic polymer condenses the nucleic acid into a complex and enters the cell through endocytosis [153]. Meanwhile, various peptides can also deliver mRNA to cells, relying primarily on cationic or amphiphilic amine groups in the peptide main and side chains, which exhibit static binding to the mRNA and form nanocomplexes [154].

3.2. Development of mRNA vaccines for COVID-9

Currently, two vaccine candidates, i.e., BNT162b2 and mRNA-1273 developed by Pfizer and Modena respectively, were granted emergency use authorization (EUA) in 2020 by the Food and Drug Administration (FDA) for the prevention COVID-19 [10,155]. The data from phase III clinical trials suggests that these vaccine candidates possess $>$ 90% protective efficacy against symptomatic SARS-CoV-2 infection alongside tolerable safety profiles [155–159]. As such, BNT162b2 and mRNA-1273 have been widely administered worldwide, and real-world evidence suggests that mRNA vaccines safely and effectively combat COVID-19 [10, 11, 155, 159]. Four types of mRNA vaccines are currently under development to combat COVID-19, i.e., nucleoside-modified mRNA vaccines, unmodified mRNA vaccines, circrNA vaccines, and saRNA vaccines [10,155]. The most suitable antigen target sites in these mRNA vaccine candidates include the entire surface spike (S) glycoprotein of SARS-CoV-2 or its receptor binding domain (RBD) [155], both of which are immunogenic and elicit robust

protective neutralizing antibody responses following recognition by the immune system [160,161].

Eight nucleoside-modified LNP-mRNA vaccine candidates are currently in or preclinical studies [155]. BNT162b2 and mRNA-1273 are nucleoside-modified LNP-mRNA vaccine candidates [155]. Meanwhile, only one circRNA COVID-19 vaccine candidate is in development, which has not yet entered clinical trials [10,155]. Due to the closed-loop structure, circular RNA vaccine candidates are more resistant to exonuclease-mediated degradation than linear mRNAs [162]. Currently, eight saRNA vaccine candidates are in clinical trials to investigate their safety and efficacy [10,155]. Meanwhile, although there are no marketed unmodified mRNA vaccines, seven candidates are being investigated in clinical trials [10,155].

Although mRNA vaccine technology and the therapeutic potential of mRNA vaccines has been studied for decades, it had not previously graduated to clinical use prior to the global COVID-19 outbreak. Nevertheless, the considerable success of the SARS-CoV-2 mRNA vaccines clearly highlights the broader potential of mRNA vaccines for other infectious diseases, such as RSV, influenza, or human immunodeficiency virus (HIV).

4. RSV mRNA vaccines

Vaccines for infectious diseases are currently the most advanced application in mRNA therapeutics [11]. The principle of mRNA vaccines in viral infectious diseases has been clarified in Fig. 4. Most mRNA vaccines currently in preclinical trials and clinical use are administered as a bolus injection into the skin, muscle, or subcutaneous space, where they enter cells through a variety of pathways and translate and express antigens that are displayed to T and B cells [11,163] (Fig. 4).

4.1. The path to RSV mRNA vaccine development

Approximately 20 years ago, a study immunized mice with recombinant Semliki Forest virus RNA, which encodes RSV F protein (Table 1). Serological analysis showed that the vaccination elicited an antigen-specific antibody response and induced a Th1 immune response [164]. This study was the beginning of RSV mRNA vaccine research. Due to instability both in vivo and in storage, and due to the difficulties of mass production, development of RSV mRNA vaccines was considered unrealistic. In 2012, researchers developed a self-amplifying RNA vaccine, using an LNP vector targeting F protein of RSV [165] (Table 1). This technology elicited broad, potent, and protective immune responses comparable to a viral delivery technology, without the inherent limitations of viral vectors [165]. In 2014, another alternative nonviral delivery system was reported based on a cationic nanoemulsion (CNE), which could bind to mRNA and enhance its delivery, thereby substantially increasing vaccine potency [166]. This delivery system was then used to deliver RSV mRNA, exhibiting a well-established safety profile, and good tolerance in children and adults, including the elderly [166] (Table 1). Meanwhile, mice immunized with a SAM-based RSV mRNA vaccine were found to induce early and intense responses to type I IFN and IFN stimulation at the injection site, which restricted the expression of antigen encoded by the original SAM [167]. The study by Pepini suggested that reduction of early type I IFN responses should be considered for subsequent development of RSV mRNA vaccines [167] (Table 1). In 2020, RSV mRNA vaccine research made a major breakthrough. Espeseth AS et al. evaluated the immunogenicity, safety, and protection against RSV challenge of LNP-encapsulated chemically modified mRNA vaccines encoding various forms of RSV F protein, including secreted, membrane-associated, prefusion-stabilized, and non-stabilized structures in rodent models [168]. These vaccine candidates, expressing either prefusion stabilized or native forms of RSV F protein, elicited robust neutralizing antibody responses in mice and

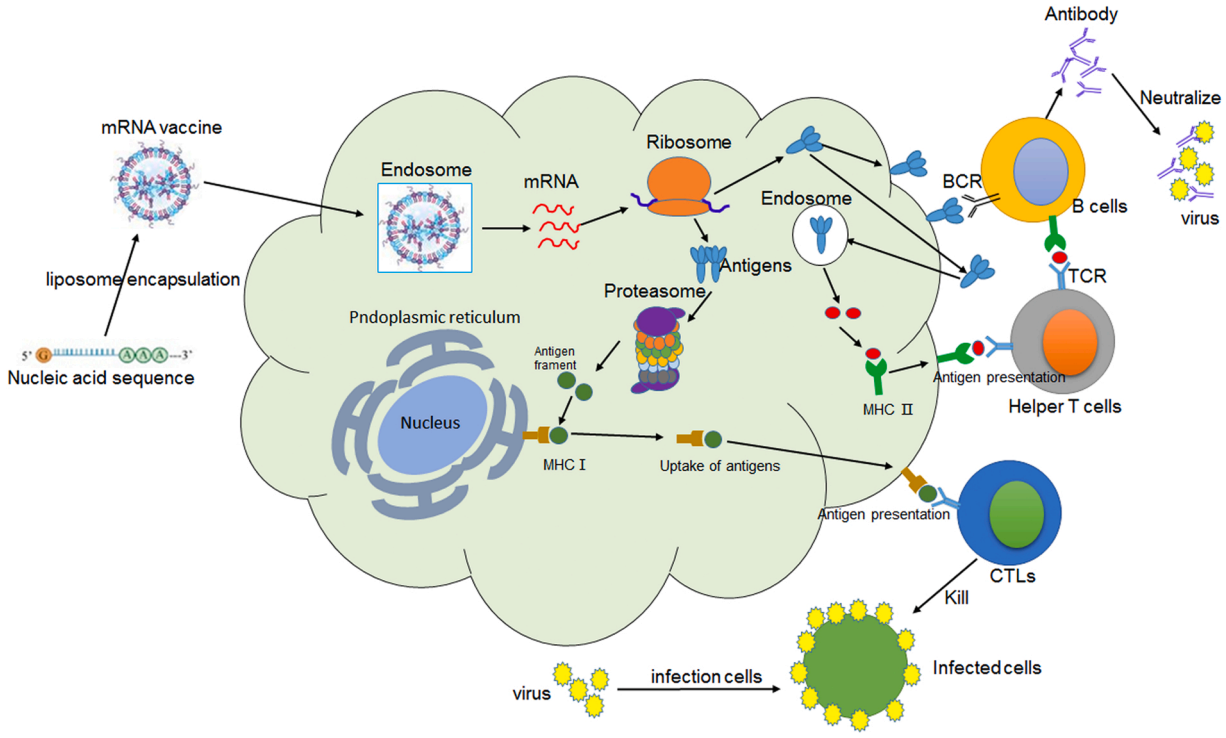

Fig. 4. Principle of mRNA vaccines viral in infectious diseases. Antigen-encoding mRNAs are formulated into LNPs, and are endocytosed in to the cells. In the cells, the mRNA were released through endosomal. The antigens are produced by the translational machinery of the cells, degraded by proteasomes, and presented on MHC class I, leading to a $CD8^+$ cytotoxic T cell response against virus. Antigens can also be anchored to the membrane of the cells and recognized by BCRs, which further lead to B cell activation. Finally, the antigens can be exported from the cells and endocytosed back to the same or another cells, degraded by endosomal proteases, and presented on MHC II structures resulting in a $CD4^+$ helper T cell response.

Table 1
Preclinical testing of mRNA vaccines against RSV.

	Target	Vaccine type	Vector	Route of administration	Conclution
1	RSV F protein	Self-replicative RNA vaccines	Semliki Forest virus	Intramuscular injection	Antigen-specific antibody responses were elicited. Predominantly Th1 type immune responses were induced.
2	RSV F protein	Self-amplifying RNA vaccine	Lipid nanoparticles	Intramuscular injection	Nonviral delivery of self-amplifying RNA encapsulated within an LNP increased immunogenicity compared with delivery of unformulated RNA.
3	RSV F protein	Self-amplifying RNA vaccine	Cationic nanoemulsion	Intramuscular injection	Nonviral delivery of self-amplifying mRNA elicits potent immune responses in mice, rats, rabbits, and nonhuman primates comparable to a viral delivery technology, and demonstrate that, relatively low doses induce antibody and T-cell responses in primates.
4	RSV F protein	Self-amplifying RNA vaccine	Lipid nanoparticles	Intramuscular injection	Minimizing the early type I IFN responses may be a useful strategy to increase primary SAM expression and the resulting vaccine potency.
5	RSV F protein , including secreted, membrane associated, pre-F-stabilized, and non-stabilized structures	Non-replicating RNA vaccine	Lipid nanoparticles	Intramuscular injection	mRNA vaccine candidates expressing either prefusion stabilized or native forms of RSV F protein elicit robust neutralizing antibody responses in both mice and cotton rats.

cotton rats, similar to the levels observed with a comparable dose of DS-Cav1. That is, cotton rats administered mRNA/LNP vaccines were fully protected against RSV-A and RSV-B challenges and did not develop ERD, unlike those immunized with FI-RSV [168]. Additionally, in contrast to DS-Cav1, mRNA vaccines elicited robust $CD4^+$ and $CD8^+$ T-cell responses, highlighting a potential advantage of the vaccines in activating cellular immune responses [168]. Together, these data suggest that mRNA/LNP vaccines, expressing forms of RSV F protein, have

Table 2
Clinical testing of mRNA vaccines against RSV.

Name	Developer	Clinical phase, trial identifier	Population type	Dose	Main goal	Antigen coding sequence	Formulation	Clinical outcome
mRNA-1777	Moderna/ Merk	Phase I, Unregistered	Healthy younger adults (ages≥18 and ≤49 years) Healthy older adults (ages≥60 and ≤79 years)	25, 100, 200 or 300 μg 25, 100, or 200 μg	Evaluated the safety, tolerability and immunogenicity of an investigational mRNA vaccine encoding the RSV F stabilized in the pre-F conformation	Pre-F protein	Nucleoside-modified mRNA-LNP	mRNA-1777 elicited a robust humoral response with RSV neutralizing antibodies, a $CD4^+$ T cell response to RSV F peptides and no serious adverse events
mRNA-1345	Moderna	Phase 1, NCT05397223	18 Years to 75 Years (Adult, Older Adult)	None	Evaluate the safety, reactogenicity and immunogenicity of modified mRNA vaccines using a systems biology approach in healthy adults	Pre- F protein	Nucleoside-modified mRNA-LNP	None
		Phase 1, NCT04528719	Younger adults (ages≥18 and ≤49 years)	50 or 100 μg	Evaluate the tolerability and reactogenicity of a single injection of up to 5 dose levels of mRNA-1345 in younger adults, women of child-bearing potential, and older adults including Japanese older adults			At month 1, the geometric mean fold rise in neutralizing antibody relative to baseline was at least 20.5 for RSV-A and at least 11.7 for RSV-B.
			Older adults (ages≥65 and ≤79 years)	50,100 or 200 μg				A single mRNA-1345 vaccination of 50, 100 or 200 μg boosted neutralizing antibody titers against RSV-A ~14-fold and RSV-B ~10-fold.
			RSV Seropositive Children (ages≥12 and ≤29 months)	None				None
			Women of Child-Bearing Potential (ages≥18 and ≤40 years)	None				None
		Phase 2, Phase 3, NCT05127434	Adults ≥ 60 Years of Age	None	Evaluate the safety and efficacy of mRNA-1345, an mRNA Vaccine targeting RSV			None
		Phase 3, NCT05330975	Adults ≥ 50 Years of Age	None	Evaluate safety, tolerability, and immunogenicity of mRNA-1345, an mRNA vaccine targeting respiratory RSV, When given alone or coadministered with a seasonal influenza vaccine.			None
mRNA-1172	Moderna	None	None	None	None	Pre-F protein	Nucleoside-modified mRNA-LNP	None
mRNA-1365	Moderna/ Merck	None	None	None	None	Pre-F protein	Nucleoside-modified mRNA-LNP	None

the potential to be safe and effective options for preventing RSV infection.

4.2. Clinical trials for mRNA vaccines against RSV

The current RSV vaccine candidates focus on targeting the highly conserved F protein as vaccinating against the pre-F conformation elicits superior neutralizing antibody responses [72]. Fortunately, mRNA vaccines can be designed to encode stabilized F protein conformations by engineering the coding sequence [168]. Indeed, mRNA vaccines encoding either the native RSV F protein or stabilized pre-F conformation were successfully delivered using cationic nanoemulsions and LNPs, without any observed instances of ERD [165, 166, 168]. Based on these studies, Moderna developed, and is currently evaluating, three single-dose vaccine candidates encoding the pre-F protein (Table 1), namely mRNA-1172 (Merck's proprietary LNPs) [11] and mRNA- 1777 (Moderna's proprietary LNPs) for adults [13], and mRNA-1345 (Moderna's proprietary LNPs) for children [15, 169–171].

The phase I trial for mRNA-1777 (V171) was conducted in Australia between November 2016 and May 2019 in healthy young adults (aged $\geq$ 18 and $\leq$ 49 years) and healthy older adults (aged $\geq$ 60 and $\leq$ 79 years) [13] (Table 2). In the phase I clinical trial, safety, tolerability, and immunogenicity of the mRNA-1777 vaccine were evaluated. Seventy two young adults were randomly assigned to receive 25, 100, or 200 μg of mRNA-1777 or placebo, and 107 older adults were randomly assigned to receive 25, 100, 200, or 300 μg of mRNA-1777 [13]. All dose levels were generally well tolerated, with no serious adverse events reported [13]. mRNA-1777 vaccination increased the humoral immune response, as validated by detecting the RSV neutralizing antibody titer, serum RSV pre-fusion F protein antibody titer, competitive antibody titer of D25 to RSV pre-fusion F protein, and cell immune response to RSV-F polypeptide [13]. T cell responses associated with RSV-F protein were also increased in the vaccine group, and was primarily related to $CD4^+$ T cell subsets, in a dose-independent manner [13]. Safety assessment found that within 7 days of vaccination, 94.4% of young volunteers and 96.3% of older volunteers reported treatment-related adverse reactions, with the most common being pain and tenderness at the injection site, followed by fatigue, generalized myalgia, and headache. Most of the adverse reactions resolved by day 8 [13].

Moderna's mRNA-1345 vaccine, which targets the RSV pre-F glycoprotein, has passed both phase I and II trials and is currently in phase III [15, 170, 171] (Table 2). The sequence of mRNA-1345 has been further designed and codon optimized to enhance translation and immunogenicity relative to mRNA-1777 [11,14]. The company recently shared interim data from the phase I study of mRNA-1345 in both younger adults (18–49 years) and older adults (65–79 years). Similar to mRNA-1777, mRNA-1345 has a good safety profile in both age groups. The most common local and systemic solicited adverse reaction was injection site pain, or headache, fatigue, and myalgia, and most adverse reactions occurred within 1–3 days after vaccination and resolved within 1–4 days [14]. In older adults, a single mRNA-1345 vaccination (50, 100, or 200 μg) boosted neutralizing antibody titers against RSV-A by 14-fold; and against RSV-B by 10-fold (Table). Importantly, the vaccine was well tolerated in older adults after a single mRNA-1345 vaccination of 50, 100, or 200 μg [14] (Table 2). Based on the optimistic data, Moderna received approval from FDA, granting fast-track designation for investigational single-dose mRNA-1345 vaccine against RSV in adults over 60 years of age [172]. Subsequently, a randomized, placebo-controlled, observer-blind phase II/III clinical trial for mRNA-1345 was registered on November 19, 2021 to evaluate the safety and tolerability of mRNA-1345 vaccine and to demonstrate the efficacy of a single dose in the prevention of the first episode of RSV-associated lower respiratory tract disease compared to that in placebo from 14 days post-injection through 12 months [171]. On February 22, 2022, the phase II clinical data of mRNA-1345 were reviewed, and a phase III randomized, observer-blind study was officially opened to evaluate the safety, tolerability, and immunogenicity of mRNA-1345 when administered alone or in combination with a seasonal influenza vaccine in adults $\geq$ 50 years of age [15]. In addition, Moderna is currently evaluating another single-dose vaccine candidate encoding the pre-F protein, mRNA-1172, in adults. mRNA-1172, which uses Merck's lipid nanoparticles as a carrier, entered phase I development in 2019 [172,173]. Moderna recently announced that mRNA-1365 encodes RSV pre-F glycoprotein and human hemipulmonary virus F protein, and is expected to enter clinical development in the near future [174]. However, currently, there is very little information regarding mRNA-1172 and mRNA-1365.

Taken together, these findings indicate that mRNA-1777 and mRNA-1365 induce a strong humoral response with RSV neutralizing antibodies without serious adverse events. At 1 month post-inoculation, the neutralizing antibody titer produced by mRNA-1345 was approximately eight times that of mRNA-1777. Hence, the available results suggest the promising prospects of mRNA vaccine for the prevention and treatment of RSV infection.

5. Challenges and trends in RSV mRNA vaccines

Current clinical trials are primarily aimed at adults and the elderly; however, data are still lacking for verification of the efficacy and tolerability of RSV mRNA vaccines in infants and young children. Fortunately, Moderna is currently recruiting subjects for a phase I to III clinical trial to evaluate tolerability and protective efficacy of the mRNA vaccines in these populations.

A significant challenge in the development of RSV vaccines is the need to demonstrate vaccine safety. Early studies evaluating FI-RSV vaccination in naïve infants established the potential risk of ERD, leading to significant increase in hospitalization and death following RSV infection. In contrast, mRNA vaccines are considered safe as they do not contain pathogenic components of the virus. Moreover, unlike DNA vaccines, mRNA does not integrate into the host genome, thereby ruling out concerns regarding insertional mutagenesis. In fact, in recent preclinical and clinical studies, no serious adverse events were observed in any of the subjects. More specifically, in the mRNA-1777 and mRNA-1345 trials, the most common adverse events were mild pain and tenderness at the injection site, whereas common systemic adverse events comprised mild headache and systemic myalgia. However, certain issues remain. For example, differences have been noted in the activation of immune responses following mRNA vaccination in animal models and humans. That is, mRNA vaccines have been shown to activate both humoral and cellular immunity, as confirmed in preclinical studies of mRNA/LNP vaccine candidates expressing RSV pre-F protein. However, in the phase I clinical trial of RSV mRNA vaccine, T cell responses were only slightly increased in the vaccine group, and the induced immune response was biased toward $CD4^+$ T cells, which was inconsistent with the results of strong $CD4^+$ T and $CD8^+$ T immune responses observed in animal models [13,168]. Moreover, mRNA vaccines may cause local and systemic inflammation [175], and potential toxicity may occur due to non-native nucleotides and vector-introduced components. Indeed, a recent study showed that an LNP-delivered mRNA vaccine induced interleukin (IL)− 1-mediated cytokine release syndrome [176]. Although injection of the mRNA vaccine in a mouse model resulted in rapidly increased expression of IL-1Rα, which is antagonistic to inflammation, in a phase I clinical data, a more pronounced increase in pro-inflammatory IL-1β was observed [176]. This reflects the differences between preclinical and clinical data. Another challenge is the strict storage and transportation conditions that are the key factors restricting the large-scale application of mRNA vaccines. mRNA vaccines often require production and distribution of frozen transportation chains, which is difficult to achieve in large-scale production in economically underdeveloped countries and regions. Nevertheless, mRNA vaccines also offer several advantages over conventional vaccines. For instance, a single mRNA vaccine can encode multiple

antigens, thus strengthening the immune response against virus infection. Therefore, subsequent RSV mRNA vaccines may be designed to contain pre-F, G protein, N protein, and other antigens to improve the immunogenicity of the vaccine. Moreover, a single mRNA vaccine can enable the targeting of multiple microbes or viral variants using a single formulation. For example, Moderna aims to integrate mRNA-1345 with its pediatric human metapneumovirus/parainfluenza virus type 3 candidate mRNA-1653 to facilitate the vaccination of children against three distinct pathogens with a single formulation. Moderna also plans to offer a triple vaccine booster against COVID-19, influenza, and RSV. RSV mRNA vaccines that have entered the stage of clinical evaluation are all non-replicative, using LNP delivery vectors. In addition, CNE delivery vectors have shown great development potential in preclinical studies [166], and should be considered in subsequent RSV mRNA vaccine development. Moreover, whether intramuscular mRNA immunization can induce meaningful neutralizing antibodies in the nasal tissue is controversial [177,178]. A recent study indicated mucosal booster vaccination was needed to establish robust sterilizing immunity in the respiratory tract against SARS-CoV-2 [179], indicating that mucosal booster vaccination also should be considered in subsequent RSV mRNA vaccine development.

6. Concluding remarks

The rapid development of new mRNA vaccines has brought hope to the field of RSV vaccine research and development. Their successful application in the prevention and control of COVID-19 has further fueled research on mRNA vaccines. Decades of progress in mRNA design and nucleic acid delivery technology, together with the discovery of novel antigen targets, have made mRNA vaccines an extraordinary tool for combating emerging pandemics and existing infectious diseases. The first two mRNA vaccines (mRNA-1345 and mRNA-1777), which were developed at revolutionary speed to fight RSV, have exceeded expectations, and may achieve complete control of RSV infection. The resultant positive safety and efficacy data, together with a proven path to regulatory approval, leave us optimistic about the potential of mRNA therapeutics for the prevention of RSV infection. Moreover, certain traditional vaccine platforms, such as live-attenuated vaccines, LID ΔM2–2, subunit vaccines, DS-CAV1, RSVpreF3, RSVpreF,vector-based vaccines, Ad26. RSV.preF, MVA-BN-RSV, and monoclonal antibodies, nirsevimab, clesrovimab, have also provided encouraging results in terms of immunogenicity and safety. Taken together, the current review has summarized the recent status of RSV mRNA vaccines, and improved the overall understanding of the latest developments in RSV vaccines.

Declarations of interest

The authors declare no conflicts of interest that pertain to this work.

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